A Spleen Diet

In a recent article by Exnecia (n. ed. Glatt domesticus).

D. N. Mohanty,

1961
"The Role of Vitamin A Deficient Diet In Coccidiosis Caused by Eimeria tenella In Domestic Fowl (Gallus domesticus)".

A Thesis
Submitted to the Faculty of Veterinary Science, Magadh University, In Partial fulfilment of the Requirements For the Degree of Master of Science (Veterinary)

PS/190

By
D. N. Mohanty,
1964
ABSTRACT

Title: "The Role of vitamin A deficient diet in Coccidiosis caused by Eimeria tenella in domestic fowl, (Gallus domesticus)."

This Thesis is the outcome of experimental studies on the role of vitamin A deficient diet in coccidiosis due to Eimeria tenella Railliet and Lucet, 1891 in chicks after they were exposed to infection. Incidentally observations were also made on certain biological aspects like sporulation and biometry of E. tenella oocysts during the course of the experiments, which are also described.

E. tenella oocysts were collected from chicks died of caecal coccidiosis. Sporulation of oocysts in different strengths of freshly prepared Potassium dichromate and formalin was studied at 23.5± 3.5C. 2% Pot. dichromate solution with frequent aeration yielded highest percentage of sporulation. Complete sporulation of oocysts was observed in as early as 13 hours. Biometric studies (length and width) of 500 oocysts, one hundred each at unsporulated and other four stages of sporulation were made. A significant difference was observed between different stages during sporulation.

Observations were made on two groups of laboratory-raised 21 days old chicks, one on basal diet and the other on vitamin A deficient diet, after exposure to 8,000
ABSTRACT

Title: "The Role of vitamin A deficient diet in coccidiosis caused by Eimeria tenella in domestic fowl, (Gallus domesticus)".

This Thesis is the outcome of experimental studies on the role of vitamin A deficient diet in coccidiosis due to Eimeria tenella Railliet and Lucet, 1891 in chicks after they were exposed to infection. Incidentally observations were also made on certain biological aspects like sporulation and biometry of E. tenella oocysts during the course of the experiments, which are also described.

E. tenella oocysts were collected from chicks died of caecal coccidiosis. Sporulation of oocysts in different strengths of freshly prepared Potassium dichromate and formalin was studied at 28.5± 3.5C. 2% Pot. dichromate solution with frequent aeration yielded highest percentage of sporulation. Complete sporulation of oocysts was observed in as early as 18 hours. Biometric studies (length and width) of 500 oocysts, one hundred each at unsporulated and other four stages of sporulation were made. A significant difference was observed between different stages during sporulation.

Observations were made on two groups of laboratory-raised 21 days old chicks, one on basal diet and the other on vitamin A deficient diet, after exposure to 3,000
sporulated oocysts of *E. tenella*. Bloody discharge was seen in both the infected groups on the 5th, 6th, and 7th days of infection. The infection was more severe in the chicks on vitamin A deficient diet than those on basal diet, as judged by mortality-rate, haemorrhage into caeca and greater elimination of oocysts and body-weight-gain after recovery.

Haematological studies were also made at weekly intervals after infection and a reduction in red cell count, haemoglobin percentage, packed cell volume and an increase in erythrocyte sedimentation rate and blood chloride value was noticed. A marked heterophilia and lymphopaenia was observed during the acute phase and eosinophilia during the recovery phase. Changes in blood picture were more marked in chicks on deficient diet than those on basal diet. Eosinophilia was more pronounced in the latter group.

Chicks kept as control on basal diet without infection weighed heavier, showed no change in blood picture during the entire period of the experiment.

Histopathological sections of caeca from both the infected groups were studied. The second generation schizonts were larger in number and size in
chicks on vitamin A deficient diet. than the other infected group on basal diet. Second generation schizonts was found to develop very close to the sub-mucosa resulting in extensive haemorrhage and tissue destruction. Similar changes were seen in caeca of chicks died in a natural out-break.
CONTENTS

ACKNOWLEDGMENTS.

INTRODUCTION. ... ... 1 - 5

REVIEW OF LITERATURE. ... ... 6 - 16

MATERIALS & METHODS. ... ... 17 - 29

OBSERVATIONS & DISCUSSION. ... ... 30 - 48

CONCLUSIONS. ... ... 49 - 50

SUMMARY. ... ... 51 - 54

REFERENCES ... ... (i) - (iv)

APPENDIX OF TABLES ... Tables I to XI.

-xxx-
CONTENTS

ACKNOWLEDGMENTS

INTRODUCTION ... ... 1 - 5

REVIEW OF LITERATURE ... ... 6 - 16

MATERIALS & METHODS ... ... 17 - 29

OBSERVATIONS & DISCUSSION ... ... 30 - 48

CONCLUSIONS ... ... 49 - 50

SUMMARY ... ... 51 - 54

REFERENCES ... ... (i) - (iv)

APPENDIX OF TABLES ... Tables I to XI.
ACKNOWLEDGMENTS.

I express my deep sense of gratitude and indebtedness to Dr. A.K. Varma, Ph.D. (London), Director, Livestock Research Station, Bihar, Patna, and Professor & Head of the Post-graduate Department of Parasitology, Bihar Veterinary College, under whose guidance and supervision this work was carried out and the Thesis entitled "The Role of Vitamin A deficient diet in Coccidiosis caused by Eimeria tenella in the domestic fowl, Gallus domesticus" prepared.

I have pleasure in recording my gratefulness to Dr. H. Prasad, Ph.D. (London), Research Officer (Poultry), Livestock Research Station, Bihar, Patna, and now Deputy Director of Animal Husbandry, Patna, for his critical suggestions given from time to time during the course of this work.

Thanks are due to Mr. R.K. Chaudhuri, B.V. Sc. & A.H., D.T. V.M. (Edin.), for his kind help in day-to-day laboratory work.

I further record my gratefulness to Mr. S.A. Ahmed, B.Sc. (Ag.), M.R.C.V.S., Principal, Bihar Veterinary College, Patna, for providing necessary facilities to pursue this work.

I am grateful to Mr. P.B. Kuppuswamy, Professor of Pathology, Bihar Veterinary College, for providing facilities to carry out histopathological work in his laboratory.

The help of Mr. J.N. Prasad, Research Officer (Statistics), Livestock Research Station, Bihar, Patna, in analysing the statistical data and that of Mr. Raghubans Bhagab, Artist in Photography, is also duly acknowledged with great appreciation.

... D.N. MOHANTY.
INTRODUCTION.

Very little was known until 1929 about Caecal Coccidiosis. Today it is a much studied and widely known disease of poultry. *Eimeria tenella* Railliet & Lucet, 1891, the causal organism of the disease is a pronounced epithelial parasite. During its life cycle, it penetrates into the caecal epithelium causing haemorrhage and death. In the past few years considerable research work has advanced our knowledge of epidemiology and the pathogenesis of Coccidiosis and revealed new therapeutic approaches though none have proved satisfactory. Nevertheless, the incidence of caecal coccidiosis is on the rise and continues to remain as one of the greatest scourges to broiler industry. The loss sustained by the poultry industry through death and little-known morbidity is enormous.

The transition from extensive to intensive rearing system has already affected the control of the disease. Any parasitic load insidiously undermines the well being of the birds and in the long run its productivity, which is so earnestly being aimed at. A limit has reached where not only the control of Coccidiosis has become more complicated but species hitherto known to be of little pathogenicity also pose a serious threat to poultry health.
INTRODUCTION.

Very little was known until 1929 about Caecal Coccidiosis. Today it is a much studied and widely known disease of poultry. *Eimeria tenella* Railliet & Lucet, 1891, the causal organism of the disease is a pronounced epithelial parasite. During its life cycle, it penetrates into the caecal epithelium causing haemorrhage and death. In the past few years considerable research work has advanced our knowledge of epidemiology and the pathogenesis of Coccidiosis and revealed new therapeuetic approaches though none have proved satisfactory. Nevertheless, the incidence of caecal coccidiosis is on the rise and continues to remain as one of the greatest scourges to broiler industry. The loss sustained by the poultry industry through death and little-known morbidity is enormous.

The transition from extensive to intensive rearing system has already affected the control of the disease. Any parasitic load insidiously undermines the well being of the birds and in the long run its productivity, which is so earnestly being aimed at. A limit has reached where not only the control of Coccidiosis has become more complicated but species hitherto known to be of little pathogenicity also pose a serious threat to poultry health.
It has been observed that diet plays a significant role in the course and development of Coccidiosis but the precise factors and the exact manner in which nutrition influences is still obscure. Several workers have stressed the importance of vitamins in Coccidiosis. The greatest importance probably lies with Vitamin A. Therefore, a few years back the birds kept on free range system usually had an uncontrolled access to green food containing provitamins, chiefly carotene and xanthophyll. These two are the precursors of vitamin A, the only vitamin produced by animal through metabolism.

Vitamin A is a MUST for the maintenance of health and growth and is vitally needed for livestock including poultry Seifried (1930) and Cruickshank (1935) reported that vitamin A is necessary for maintaining the normal structure of epithelial linings. They further reported that the deficiency of this vitamin changes the normal epithelium to stratified keratinizing epithelium which adversely affected the natural protective function of the cells and disturbed the absorption of nutrients. The lack of bactericidal mucus in hypo-vitaminosis A according to Seifried (1935) results in an increase in the number of saprophytes
It has been observed that diet plays a significant role in the course and development of Coccidiosis but the precise factors and the exact manner in which nutrition influences is still obscure. Several workers have stressed the importance of vitamins in Coccidiosis. The greatest importance probably lies with Vitamin A. Therefore, a few years back the birds kept on free range system usually had an uncontrolled access to green food containing provitamins, chiefly carotene and xanthophyll. These two are the precursors of vitamin A, the only vitamin produced by animal through metabolism.

Vitamin A is a MUST for the maintenance of health and growth and is vitally needed for livestock including poultry Seifried (1930) and Cruickshank (1935) reported that vitamin A is necessary for maintaining the normal structure of epithelial linings. They further reported that the deficiency of this vitamin changes the normal epithelium to stratified keratinizing epithelium which adversely affected the natural protective function of the cells and disturbed the absorption of nutrients. The lack of bactericidal mucus in hypo-vitaminosis A according to Seifried (1935) results in an increase in the number of saprophytes
in the intestine and such disturbances in the microflora reduce the host resistance rendering it vulnerable to infections like Coccidiosis. The same author in 1935 was able to demonstrate that this local susceptibility to infection was reversible. After administration of vitamin A the keratinized epithelium is regenerated to form normal functional epithelium resorting natural resistance to infections. It is, therefore, evident that through a healthy epithelium, which is so adversely affected in hypovitaminosis A, pathogens cannot gain an entry easily.

From the literature available, it appears that very little information is available as to the specific effects of vitamins in Coccidiosis. Controversial reports are at hand about the beneficial role played by vitamin A in the course of E. tenella infection. Curiously enough under ordinary farm conditions most outbreaks of Coccidiosis occur in summer months when there is acute shortage of greens.

It is well known that the intensity of parasitic disease is correlated with the leucocytic picture and other haematological changes. Therefore, present trend has been to analyse the various aspects
of the disease. And such examples are available in which the changes in blood following profuse haemorrhage into the caecal bag on 5th, 6th and 7th days have been studied by various workers in chicks getting normal diet with liberal supply of either vitamin A or its precursors.

With little available information as to the role played by vitamin A in relation to caecal Coccidiosis, it was thought desirable to make a further reappraisal of the subject on a comparative basis in normal and vitamin A deficient chicks on the following aspects:

1. Some aspects of biology of *E. tenella* pertaining to sporulation time, appropriate media for culturing the oocysts and some biometrical data (length and width) of oocysts during various stages of sporulation.

2. Role of vitamin A in the control of Coccidiosis with particular reference to mortality rate, haemorrhage, oocyst elimination and growth rate after recovery.

3. Haematological studies during acute phase and after recovery from the disease.

4. Histopathological studies of infected caeca.
of the disease. And such examples are available in which the changes in blood following profuse haemorrhage into the caecal bag on 5th, 6th and 7th days have been studied by various workers in chicks getting normal diet with liberal supply of either vitamin A or its precursors.

With little available information as to the role played by vitamin A in relation to caecal coccidiosis, it was thought desirable to make a further reappraisal of the subject on a comparative basis in normal and vitamin A deficient chicks on the following aspects:

1. Some aspects of biology of *E. tenella* pertaining to sporulation time, appropriate media for culturing the oocysts and some biometrical data (length and width) of oocysts during various stages of sporulation.

2. Role of vitamin A in the control of coccidiosis with particular reference to mortality rate, haemorrhage, oocyst elimination and growth rate after recovery.

3. Haematological studies during acute phase and after recovery from the disease.

4. Histopathological studies of infected caeca.
5.

Work on these aspects has been described and discussed in this thesis in the light of previous works; and the results on statistical data pertaining to length and breadth of the oocysts of *E. tenella* furnish a new kind of information.
Work on these aspects has been described and discussed in this thesis in the light of previous works; and the results on statistical data pertaining to length and breadth of the oocysts of *E. tenella* furnish a new kind of information.
REVIEW OF LITERATURE.

1. **BIOLOGY:**

The sporulation time reported for most species of coccidia listed by Becker (1952) was based on observations at varying room temperatures. The sporulation time for *E. tenella* oocysts is given as 48 hours. Edgar (1955) reported that *E. tenella* oocysts sporulated best at 29 ± 1°C. They sporulated to infective stage earliest in 18 hours. Several workers (Jankiewicz & Scofield, 1934) have reported that 2% - 2.5% Potassium dichromate solution is a suitable media for culturing the oocysts. In fact this is employed as routine media, in all laboratories.

Edgar (loc. cit.) reported that the mean measurement of *E. tenella* oocyst in microns was 22 x 19. Becker (loc. cit.) gives an average value of 22.6 x 19 μ with a range of 19.5 - 26 μ (length) and 16.5 - 22.8 μ (width) for *E. tenella*. Levine (1961) gives a mean value of 22.9 by 19.1 μ, the range being 14 - 31 by 9 - 25 μ. Becker, Zimmerman and Patillo (1955) made biometric study of *E. brunetti* oocysts. They reported that the dimensions would probably not change significantly during the sporulation process. They further suggested that the average size of oocysts would depend, among other factors, on the time the sample
was taken during the patent period as well as on certain undermined factors in the individual host. A search of available literature could reveal no biometric study of *E. tenella* oocysts.

II. **ROLE OF VITAMIN A IN CAECAL COCCIDIOSIS.**

There are several reports stating the relationship between vitamin A content of the diet and coccidiosis. Tyzzer (1929) noticed weakness in legs of chicken reared under laboratory conditions and incorporated cod liver oil in the ration. This probably would have influenced the course of experimental caecal coccidiosis. Allen (1932) noted that chicken of the same age maintained under practically identical conditions on two different types of ration, one having a higher protein content, 40% more vitamin A and about 20% more vitamin B (complex) than the other, when infected with an approximately same number of *E. tenella* oocysts, the following correlation was observed. The high-protein or high-vitamin ration was directly or indirectly responsible for the escape from the severe acute coccidiosis. There was little haemorrhage of shorter duration and the mortality rate was low. This phenomenon was regarded as a mechanism for producing immunity. A reverse effect was noticed in chicken fed with low protein and low vitamin diet.
was taken during the patent period as well as
on certain undermined factors in the individual
host. A search of available literature could
reveal no biometric study of *E. tenella* oocysts.

II. **ROLE OF VITAMIN A IN CAECAL COCCIDIOSIS.**

There are several reports stating the
relationship between vitamin A content of the diet
and coccidiosis. Tyzzer (1929) noticed weakness in
legs of chicken reared under laboratory conditions
and incorporated cod liver oil in the ration.
This probably would have influenced the course of
experimental caecal coccidiosis. Allen (1932) noted
that chicken of the same age maintained under
practically identical conditions on two different
types of ration, one having a higher protein content,
40% more vitamin A and about 20% more vitamin B
(complex) than the other, when infected with an
approximately same number of *E. tenella* oocysts,
the following correlation was observed. The high-
protein or high-vitamin ration was directly or in-
directly responsible for the escape from the severe
acute coccidiosis. There was little haemorrhage
of shorter duration and the mortality rate was low.
This phenomenon was regarded as a mechanism for
producing immunity. A reverse effect was noticed in
chicken fed with low protein and low vitamin diet.
was taken during the patent period as well as on certain undermined factors in the individual host. A search of available literature could reveal no biometric study of E. tenella oocysts.

II. **ROLE OF VITAMIN A IN CAECAL COCCIDIOSIS.**

There are several reports stating the relationship between vitamin A content of the diet and coccidiosis. Tyzzer (1929) noticed weakness in legs of chicken reared under laboratory conditions and incorporated cod liver oil in the ration. This probably would have influenced the course of experimental caecal coccidiosis. Allen (1932) noted that chicken of the same age maintained under practically identical conditions on two different types of ration, one having a higher protein content, 40% more vitamin A and about 20% more vitamin B (complex) than the other, when infected with an approximately same number of E. tenella oocysts, the following correlation was observed. The high-protein or high-vitamin ration was directly or indirectly responsible for the escape from the severe acute coccidiosis. There was little haemorrhage of shorter duration and the mortality rate was low. This phenomenon was regarded as a mechanism for producing immunity. A reverse effect was noticed in chicken fed with low protein and low vitamin diet.
Jones (1934) on the other hand reported that there is no difference in the course of E. tenella infection which could be attributed to vitamin A content of the diet. The haemorrhage and mortality were not constantly correlated with the diet. Murphy, Hunter and Knandel (1938) showed that fish liver oil exerted a favourable influence on the course of coccidiosis. It hastened recovery from coccidiosis. Jungherr (1945) observed that there is relationship between a-vitaminosis A and occurrence of caecal coccidiosis. Taylor and Russell (1946) expressed the opinion that when conditions favour incidence of caecal coccidiosis the severity of the disease is accentuated in chicks deficient in vitamin A. Davies (1952) observed that vitamin A content of liver fell to a lower level in chicks spontaneously affected with coccidiosis. Schoop, Wagner and Minner (1954) observed that a deficiency of vitamin A affected the chicks adversely, predisposing them to coccidiosis. Wachendorfer (1957) confirmed this finding. Gylostroff (1959) observed that in large flocks under field conditions vitamin A was capable of influencing the course of coccidiosis. Erasmus, Scott and Levine (1960) noted that the severity of coccidiosis was similar in chicks receiving the
Jones (1934) on the other hand reported that there is no difference in the course of *E. tenella* infection which could be attributed to vitamin A content of the diet. The haemorrhage and mortality were not constantly correlated with the diet. Murphy, Hunter and Knandel (1938) showed that fish liver oil exerted a favourable influence on the course of coccidiosis. It hastened recovery from coccidiosis. Jungherr (1945) observed that there is relationship between a-vitaminosis A and occurrence of caecal coccidiosis. Taylor and Russell (1946) expressed the opinion that when conditions favour incidence of caecal coccidiosis the severity of the disease is accentuated in chicks deficient in vitamin A. Davies (1952) observed that vitamin A content of liver fell to a lower level in chicks spontaneously affected with coccidiosis. Schoop, Wagner and Minner (1954) observed that a deficiency of vitamin A affected the chicks adversely, predisposing them to coccidiosis. Wachendorfer (1957) confirmed this finding. Gylstroff (1959) observed that in large flocks under field conditions vitamin A was capable of influencing the course of coccidiosis. Erasmus, Scott and Levine (1960) noted that the severity of coccidiosis was similar in chicks receiving the
vitamin, recovery of surviving chicks as measured by improved appetites and growth rate, was increased. Gerriets (1960 & 1961) confirmed the earlier work of Schoop et al. (loc. cit.) and observed that vitamin A supplements influenced favourably the course of caecal coccidiosis. Vitamin A deficiency appeared to increase the susceptibility. He recommended a combination of vitamin A with coccidiostatic drugs for control of the disease. Pellérdy (1962) observed that a single administration of 5,000 to 10,000 i.u. vitamin A is sufficient to reduce the extent and severity of coccidiosis. In all his experiments the losses appeared later and the outbreak was less severe in the treated group. He further observed that higher doses of vitamin A did not seem to be either of advantage or disadvantage and it was not effective against massive infection.

Waldroup et al. (1963) noticed no significant difference in mortality or weight gain of chicks given 30,000 sporulated oocysts of E. tenella at 21 days of age kept on ration poor in vitamin A or containing up to 1,710 i.u. vitamin A. Joyner (1963) states that the diet of the host can influence the coccidial infection and reduce the mortality in chicks.
infected with *E. tenella*. He further reports that vitamin A and K have beneficial effects. Panda *et al.* (1962 & 1964) working on the effect of vitamin A in *E. acervulina* and *E. necatrix* infection, observed that higher intake of vitamin A tended to minimise directly or indirectly the adverse effect of coccidiosis on growth.

(a) **Clinical symptoms:**

Tyzzer (*loc. cit.*) in his classical report on "Coccidiosis in Gallinaceous birds" states that: "Chickens experimentally infected through ingestion of large numbers of oocysts appear normal until the 4th day of infection when they become less active, eat little, and seek the header for warmth. If killed at this time, beginning haemorrhage is found throughout the caecal mucosa. On the following day, five days after the infective feeding, there are copious intestinal discharges of bright red blood which frequently becomes smeared over the feathers posteriorly and the exposed skin and the mucous membranes appear pale. Death may occur at this time or on the following day, when post mortem examination shows profuse haemorrhage from the caecal mucosa, in some instances also from that
Jones (1934) on the other hand reported that there is no difference in the course of *E. tenella* infection which could be attributed to vitamin A content of the diet. The haemorrhage and mortality were not constantly correlated with the diet. Murphy, Hunter and Knandel (1938) showed that fish liver oil exerted a favourable influence on the course of coccidiosis. It hastened recovery from coccidiosis. Jungherr (1945) observed that there is relationship between a-vitaminosis A and occurrence of caecal coccidiosis. Taylor and Russell (1946) expressed the opinion that when conditions favour incidence of caecal coccidiosis the severity of the disease is accentuated in chicks deficient in vitamin A. Davies (1952) observed that vitamin A content of liver fell to a lower level in chicks spontaneously affected with coccidiosis. Schoop, Wagner and Minner (1954) observed that a deficiency of vitamin A affected the chicks adversely, predisposing them to coccidiosis. Wachendorfer (1957) confirmed this finding. Gyllenstroff (1959) observed that in large flocks under field conditions vitamin A was capable of influencing the course of coccidiosis. Erasmus, Scott and Levine (1960) noted that the severity of coccidiosis was similar in chicks receiving the
infected with *E. tenella*. He further reports that vitamin A and K have beneficial effects. Panda *et al.* (1962 & 1964) working on the effect of vitamin A in *E. acervulina* and *E. necatrix* infection, observed that higher intake of vitamin A tended to minimise directly or indirectly the adverse effect of coccidiosis on growth.

(a) **Clinical symptoms:**

Tyzzer (*loc. cit.*) in his classical report on "Coccidiosis in Gallinaceous birds" states that:

"Chickens experimentedally infected through ingestion of large numbers of oocysts appear normal until the 4th day of infection when they become less active, eat little, and seek the hover for warmth. If killed at this time, beginning haemorrhage is found throughout the caecal mucosa. On the following day, five days after the infective feeding, there are copious intestinal discharges of bright red blood which frequently becomes smeared over the feathers posteriorly and the exposed skin and the mucous membranes appear pale. Death may occur at this time or on the following day, when post mortem examination shows profuse haemorrhage from the caecal mucosa, in some instances also from that
infected with *E. tenella*. He further reports that vitamin A and K have beneficial effects. Panda et al. (1962 & 1964) working on the effect of vitamin A in *E. acervulina* and *E. necatrix* infection, observed that higher intake of vitamin A tended to minimise directly or indirectly the adverse effect of coccidiosis on growth.

(a) **Clinical symptoms:**

Tyzzer (loc. cit.) in his classical report on "Coccidiosis in Gallinaceous birds" states that chickens experimentally infected through ingestion of large numbers of oocysts appear normal until the 4th day of infection when they become less active, eat little, and seek the shelter for warmth. If killed at this time, beginning haemorrhage is found throughout the caecal mucosa. On the following day, there are copious intestinal discharges of bright red blood which becomes smeared over the feathers posteriorly. Noted exposed skin and the mucous membranes appear red. Death may occur at this time or on the following day when post mortem examination shows profuse bleeding from the caecal mucosa, in some instances also and
of large intestine and areas of the lower small intestine, pallor of visera and tissue, and in extreme cases reduction of the blood in heart and tissues to a pinkish watery fluid. Death may be delayed in some individuals but those survive more than seven days may be expected to recover rapidly from a single infection if furnished adequate warmth and a proper diet”.

Mayhew (1933) observed bloody droppings on 4th day after infection and one bird died on the fourth day. He later (1937) observed cores in the caecum made up of tissue debris and blood corpuscles. He noticed that such cores were passed out in the faeces from about 3 to 6 days after haemorrhage in the shape of hollow tubes. About 7 days after infection the caecal wall changed colour from red to mottled reddish or milky white due to the formation of oocysts.

(b) Oocyst production: Allen (loc. cit.) reported consistent lower oocyst-production in chicken reared on high-protein and high-vitamin diet. Jones (1934) noted oocyst production on the basis of number of oocysts passed per gramme of faeces. It was observed that oocyst-production was more in chickens in high-protein diets than among low-protein diets. Herrick, Ott and Holmes (1936b) found the oocysts in droppings of infected birds up to 7½ months after infection.
The oocysts appeared in the droppings after the 7th day of infection. Wachendorff (1957) observed that the elimination of oocysts was inversely proportional to the intake of vitamin A.

(c) **Growth rate after recovery**:

Allen (loc. cit.) reported the average weight gain in chickens kept on Bureau of Animal Industry ration (high protein and high vitamin ration) and Indiana ration (low protein and low vitamin ration) after recovery. It was seen that the chickens on Indiana ration weighed heavier than the chickens on B.A.I. ration on 18th day after infection. Chickens reared on B.A.I. ration had developed chronic coccidiosis and presumably it hindered in their weight gain. Mayhew (1932a, b and 1934a) reported that the growth rate was influenced in severely affected birds. The fowls do not gain the normal weight until maturity. The recovered birds may suffer the ill effects for some time or even permanently.

Bressler and Gordenk (1951) observed that the body weight of birds which survived an outbreak of caecal coccidiosis was slightly less than the weight of birds fed with sulfamethoxaline in the mash as a preventive measure. Gardiner (1954) reports that there exists an inverse correlation between severity of the
disease and weight gain and growth. Waldroup et al. (1963) observed that high dietary levels of vitamin A fed during or prior to the infection with coccidia did not significantly influence the morbidity as measured by weight gain. Panda et al. (1964) infected chicks with *E. acervulina* and *E. necatrix* and noticed the gain of weight in moderately severe infections. They stated that when the dietary level of vitamin A was increased during the acute phase of the disease, the weight gains were significant. They further observed that all the infected groups gained slightly more rapidly than the controls. There was a significant difference in weight gains in the group which had received the lowest level of dietary vitamin A. Their study also showed that normal dietary level of vitamin A is not sufficient for speedy recovery from coccidiosis. A higher vitamin A level in diet has a definite beneficial value.

III. CLINICAL HEMATOLOGY.

Tyzzer (*loc. cit.*) reported about the reduction of blood volume in heart in extreme cases. The blood appeared to be pinkish and watery. Herrick, Ott and Holmes (1936a) obtained some important data regarding effect of age group on red blood cell count and mortality in experimentally infected cases. They observed that
the red blood cell count was normal until almost the 5th day after infection, after which it declined precipitously until 7th day. Then it slowly became normal by 27th day of infection in recovered cases. They further noted that the mortality and red blood cell decrease were heavy between 1½ to 2 months of age.

Herrick (unpublished) and Waxler (1941b) demonstrated a significant decrease in blood haemoglobin during the haemorrhagic phase. Natt and Herrick (1955) reported approximately 50% decrease in erythrocyte count per cubic millimeter of blood on the 5th and 6th days of the infection. It required eight days following the blood loss to return to normal. The haematocrit value also decreased in the same magnitude and on the same days as erythrocyte count and required approximately the same number of days to return to normal (Natt & Herrick, 1956).

Natt (1959) found changes in leucocytic picture in E. tenella infection. He observed lymphopaenia and heterophilia on the 5th day and eosinophilia on the 10th day after infection. A marked leucocytosis was observed from 7th day of infection which persisted through recovery phase.

Anaemia occurs due to loss of blood through haemorrhage in caeca. Joyner and Davies (1960) determined the haematocrit value during acute phase of
the disease. They noted a marked decrease in packed red cell volume on the 5th day of experimental infection. It returned to normal on 10th day of infection. Mincheva (1963) reported a correlation between the number of erythrocytes and the amount of haemoglobin on the one hand and the severity of caecal coccidiosis on the other. The loss of blood was proportional to the degree on caecal damage.

Waxler (1941a) stated that there was an increase of blood chloride on the 6th and 7th day of infection of caecal coccidiosis in chickens.

IV. HISTOPATHOLOGY:

Tyzzer (loc. cit.) studied the stained sections of the caeca taken at various developmental stages of *E. tenella* infection and observed that large schizonts developed in the epithelial cells of the fundi of the glands. This parasitized cell lost its identity and increased rapidly in size and became disassociated (independent entities) infiltrated the adjacent tissues while the superficial portion of the gland which was not usually invaded, healed over and regeneration took place. The growing organism largely replaced the glandular epithelium. The next phase of development, the gametocytes, were found beneath the nuclei of invaded cell, thus indicating the tendency
the disease. They noted a marked decrease in packed red cell volume on the 5th day of experimental infection. It returned to normal on 10th day of infection. Mincheva (1963) reported a correlation between the number of erythrocytes and the amount of haemoglobin on the one hand and the severity of caecal coccidiosis on the other. The loss of blood was proportional to the degree on caecal damage.

Waxler (1941a) stated that there was an increase of blood chloride on the 6th and 7th day of infection of caecal coccidiosis in chickens.

IV. HISTOPATHOLOGY:

Tyzzer (loc. cit.) studied the stained sections of the caeca taken at various developmental stages of E. tenella infection and observed that large schizonts developed in the epithelial cells of the fundi of the glands. This parasitized cell lost its identity and increased rapidly in size and became disassociated (independent entities) infiltrated the adjacent tissues while the superficial portion of the gland which was not usually invaded, healed over and regeneration took place. The growing organism largely replaced the glandular epithelium. The next phase of development, the gametocytes, were found beneath the nuclei of invaded cell, thus indicating the tendency
I. (a) **Collection of infective materials.**

*Eimeria tenella* oocysts were collected in large number from chicks, died of caecal coccidiosis, brought for postmortem examination at Livestock Research Station, Patna, (Poultry Section), on two different occasions in March, 1964. These formed the infective material. The infective brews were prepared by harvesting the caeca. The caeca were opened, the caecal cores separated, the outer surface lightly scraped all over and the scraping from the caecal wall along with the caeca were thoroughly macerated in a sterile and clean pestle and mortar with little normal saline. The suspension was then filtered through sieves. The technique employed at Weybridge laboratory was adopted with slight modification. Saturated sugar solution was substituted for salt solution for levitation of oocysts. Joyner and Davies (1960) observed that there was some degree of deterioration in oocysts separated by Sodium chloride floatation method. Disintegration of oocysts and irregular sporulation were minimised when use of salt was avoided. The suspension containing the oocysts was centrifuged at 1500 R.P.M. for one minute.
18.

The supernatent fluid was decanted and the sediment was examined, for the presence of oocysts, under the low power objective of the microscope. Saturated sugar solution was added to the sediment in the centrifuge tubes and shaken well. The tubes were again centrifuged for one minute at 1500 R.P.M. The oocysts were, thus, freed from debris. A drop from the top of the centrifuge tube was examined under microscope and large number of oocysts was seen.

(b) **Sporulation of oocysts:** A column of about 1.5 cc. from the top of the tubes, where oocysts were in maximum concentration was pipetted out and freed of sugar by repeated washing and centrifugation. The oocysts were allowed to sediment overnight in normal saline solution and centrifuged next morning. The supernatent fluid was decanted and the sediments were mixed with different strength of Potassium dichromate solution and formaline. Freshly prepared Pot. dichromate solution 2.5%, 2%, 1% and 0.5% and formaline 0.5% were used. In one case old stock of Pot. dichromate solution was used to note the effect of sporulation. The oocysts were left to sporulate in wide Petri dishes at room temperature (varying between 25 C. to 32 C. with average of 28.5 C.).
The culture media was aerated twice daily with the help of glass pipettes. Few Petri dishes were left without stirring for comparing with the effect of aforesaid oxygenation on sporulation. Distilled water was frequently added to compensate the loss through evaporation.

The percentage of complete sporulation was determined after counting a minimum of 300 oocysts. Minimum time taken for sporulation was also recorded.

(c) **Measurement of oocysts (Biometry).**

Biometrical study of oocysts at various stages of sporulation was made with a fresh culture. 500 oocysts were measured at various stages of development. The oocysts were looped on to a glass slide and immediately covered with a cover slip. They were measured with the help of the ocular micrometer calibrated against a stage micrometer previously. 10x eye piece and 40x objective was used. The same microscope was used throughout the experiment to avoid any error. Oocysts were measured in the order in which they came to view to avoid selection. The length and width of the oocysts were thus determined. The range of variations and the mean were worked out. The variations at each stage of sporulation were determined by "F" test.
Camera lucida drawings of the oocysts were made at various stages of sporulation.

II. The experiment.

A. (a) Experimental birds:

150 cross-bred day-old chicks were brought from Central Poultry Farm, Patna, on 11.4.64. The chicks were protected against Ranikhet disease with F1 strain. The chicks were divided into two groups of ninety and sixty respectively. They were reared in an electrically heated brooder which was thoroughly disinfected previously. The room had also been disinfected prior to putting in the chicks. The two groups of chicks were kept on experimental ration from the very beginning. The weight of each chick at one-day-old age was recorded. The chicks were wing-banded at the same age.

(b) Experimental feed:

Different workers have recommended basal diets and diets deficient in vitamin A content. Fundamentally all such diets are similar. Deo et al. (1962) used the following diet computed at Indian Veterinary Research Institute, Izatnagar:

**Basal diet:**

- Ground wheat ... 50 parts.
- Gram chunni ... 20 parts.
- Ground oats ... 10 parts.
21.

Groundnut oil cakes ... 19 parts.
Salt ... 1 part.
Cod liver oil ... 1 part.
Meat offal ... given as wet mash.
Greens ... Berseem ad. libitum.
Lime stone ... ad. libitum given in dry mash.

**Vitamin A deficient diet.**

Ground wheat ... 50 parts.
Gram chunni ... 20 parts.
Ground oats ... 10 parts.
Groundnut oil cake ... 19 parts.
Salt ... 1 part.
Meat offals ... mixed with wet mash.
Lime stone ... mixed with dry mash. ad. lib.

These two feeds were used throughout the experiment. Groups of 90 and 60 chicks were reared on the above diet respectively. The chicks were reared in a rat-proof room. All possible measures were taken to prevent entry of any kind of infection into the flock. The chicks were fed with respective ration four times a day. Chopped berseem from Government Cattle Farm, Patna, was fed ad. lib. to the group of
90 chicks. Feeding and water pans were regularly cleaned and fresh water supplied ad. lib.
The improvised run and the room were cleaned once a day. Weight of chicks from both groups was taken at weekly interval. Faecal samples from the chicks were examined prior to infection. The findings were negative.

(c) **Haematology:**

Blood was taken in the morning hours for haematological studies from 6 (six) one month-old chicks picked up randomly to determine normal values of blood picture. These chicks were reared on standard poultry ration supplied by the Central Poultry Farm, Patna, which consisted of the following:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>10</td>
</tr>
<tr>
<td>Fish meal</td>
<td>9</td>
</tr>
<tr>
<td>G.N. oil cake</td>
<td>20</td>
</tr>
<tr>
<td>Salt</td>
<td>2</td>
</tr>
<tr>
<td>Line stone powder</td>
<td>2</td>
</tr>
<tr>
<td>Yellow maize</td>
<td>50</td>
</tr>
<tr>
<td>Bone meal</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin supplements</td>
<td>5</td>
</tr>
</tbody>
</table>

Samples of blood were taken directly from the heart of Hofstad's technique in test tubes containing 0.02 cc. of Potassium oxalate and Ammonium oxalate.
23.

The anticoagulant was heated to dryness in a water bath at 60°C. The ratio of the two anticoagulants was as follows:

- Potassium oxalate ... 2 grammes.
- Ammonium oxalate ... 3 grammes.
- Distilled water ad. ... 100 cc.

The quantity of anticoagulant in a tube was sufficient for 1 cc. of blood. Blood smears were prepared from unoxalated blood on clean dry slides. To keep the variation in differential counts to the minimum an effort was made to make the smears as uniform as possible. The smears were stained by Lieszman's stain, modified Wright's stain and Giemsa stain. The recommended staining procedure for each technique was adopted but the best result was obtained with Lieszman's stain.

(i) Differential counts of leucocytes were made after counting 200 cells and working out average in each examination. Care was taken to determine each differential count from the same area of the blood smear and to avoid duplication in counting.

(ii) Total count of red blood corpuscles was made from each case. Diluting fluid described for the use in mammalian blood is said to be suitable for avian blood also (Coffin, 1963; Nambiar, et al., 1962).
Shastri and Dhand (1962) modified the formula of Vallarine (1941) for erythrocyte counts in veterinary practice. Hayem's diluting fluid and modified Vallarine formula were used. The later fluid was found to give better result and was adopted throughout the experiment.

The composition of the fluid is given below:

- Iodine ... 0.3 grammes.
- Pot. Iodide ... 0.4 grammes.
- Sodium citrate ... 2.0 grammes.
- Distilled water ... 100 ml.

The red blood cells took yellowish stain. There was no distortion in shape and the cells were evenly spaced in the counting chamber. Technique as described by Coffin (1953) for red cell count was adopted.

(iii) Haemoglobin percentage was estimated by Farbstab haemometer using acid haematin method. The procedure adopted was according to literature supplied with the apparatus. The hydrochloric acid dissolves out the nuclei from the erythrocytes along with the haemoglobin and thereby gives erroneously high values because of the turbidity imparted to the solution. To obviate this error, the following correction factor (Dukes and Schwarte, 1931) was applied to the haemometer reading.
Uncorrected reading x 0.91 - 1.49 = corrected reading.

(iv) Erythrocyte sedimentation rate was determined immediately after the blood was collected in tubes containing routine anticoagulants in proportionate quantity. Clean and dry Wintrobe haematocrit tubes of 11 cm. long and 2.5 mm. bore having a graduation mark in centimeters and millimeter scale were used. One such tube was filled with venous blood up to 10 cm. mark with a special loading pipette, taking care that no air bubble remained in the blood column. The tube was allowed to stand in a perfectly vertical position for two hours. The fall of red cells was recorded at the end of one hour, one-and-half hour, and two hours. The average sedimentation per hour was determined.

(v) Packed Cell Volume was determined by centrifuging the tube at 3,100 r.p.m. till a constant packed cell reading was obtained. Usually 25 to 30 minutes were sufficient for getting a constant packed cell volume.

(vi) Blood chloride content was determined as per procedure laid down in Fisher's Clinical Electrophotometer. Blood chloride was estimated in both vitamin A deficient chicks and those on normal diet.

Post-infection haematological studies were made on above lines at weekly intervals till the surviving chicks were sacrificed.
(B) (1) Establishment of infection:

Three groups of 3 week-old chicks, each consisting of 10 chicks were selected at random. The groups were named as A, B, and C. Chicks of group A were on basal diet, group B on vitamin A deficient diet and group C as control on basal diet. They were kept at room temperature in separate cages with wire netting floor to prevent reinfection due to self picking. The cages were cleaned daily. Respective ration was given four times a day and water supplied ad. lib. The feeding and watering vessels were cleaned each time, the feed was given, to prevent reinfection. The control birds were kept away from the infected ones and fed separately.

The oocysts kept in Potassium dichromate solution for experimental infection were washed by repeated centrifugation with distilled water for final concentration and resuspended in a small quantity of distilled water (about 10 - 12 ml.). Oocysts in 1 ml. of this suspension were then counted with the help of haemocytometer. Average of ten readings was taken to determine the concentration of oocysts in 1 ml. of the suspension. The percentage of sporulation was predetermined.

8,000 sporulated oocysts of Eimeria tenella were fed to each chick, in group A and group B, on 2.5.64 per os with the help of a micropipette.
Care was taken to avoid spilling of the infective dose. Group C was maintained as control without infection.

(ii) Clinical symptoms were observed daily postinfection and mortality among infected chicks was recorded. Weight of the chicks of all the three groups were taken individually at weekly interval.

(iii) Discharge of oocysts: For determining the elimination of oocysts in both the groups A and B and the control group, faecal samples were collected at random between 2 p.m. to 6 p.m. according to Prasad (1963), daily from 8th day of infection. Mc Master technique could not be adopted due to non-availability of Mc Master Slide. Stoll's method (second modification - centrifuge required, Richardson & Kendall, 1957), was adopted but this proved to be unsatisfactory for various reasons. The haemocytometer technique was, therefore, undertaken for counting of the oocysts. Oocysts per cc was determined at first and from there number of oocysts present in 0.15 cc was calculated. The number obtained was multiplied by 100 to give the oocyst count per gram of faeces. Mean of forty-eight haemocytometer readings was taken of each sample. Count was determined till faeces of surviving
chicks became negative for the presence of oocysts for three consecutive days.

III. HISTOPATHOLOGY:

Caeca from chicks died of experimental infection were preserved in 10% formosaline and Zenker’s fluid. The chicks which had recovered from the infection were kept under observation for a period of one month, when oocyst elimination was determined. At the end of the month all the surviving chicks including controls were sacrificed. The caeca were preserved for histopathological work in 10% formosaline. Few chicks from all groups including control were also sacrificed during the acute phase of the disease. The caeca were preserved for histopathological examination.

A nature outbreak of caecal coccidiosis occurred in the original stock raised in a separate room. Several chicks died and lesions on the caeca were seen on post-mortem. Death occurred both normal and deficient groups. The caeca of such cases were preserved for histopathological examination.

For histopathological work routine laboratory techniques as described in HAND BOOK OF HISTOPATHOLOGICAL TECHNIQUE by Culling (1957) was adopted. Sections of 4 to 6μ thickness were cut with Spencer’s rotary
microtome and stained by Harris' alum haematoxylin and eosin and Feulgen's method. The number and size of schizonts in histopathological section from both chicks on basal and deficient diet, infected with *E. tenella*, was determined. Natural cases were also treated on similar lines.
Camera Lucida Drawings Of *E. tenella* Oocysts During Sporulation.
Camera Lucida Drawings Of E. tenella Oocysts During Sporulation.
OBSERVATIONS AND DISCUSSION.

I. Biology:

Oocysts of *E. tenella* free from debris, after 12-hour sedimentation in normal saline, were mixed in different strengths of Potassium dichromate solution and formaline and allowed to sporulate at room temperature of 28.5 ± 3.5°C. The percentage of sporulation was determined after counting 300 oocysts. Complete sporulation of 52% oocysts was observed in 2% Pot. dichromate solution as early as 18 hours. In some cases, sporulation commenced after 19 hours. Highest percentage (92%) of sporulation was recorded in freshly prepared 2% Pot. dichromate solution. The percentage of sporulation obtained in different media after 48 hours is recorded in Table I. Incomplete sporulation was noticed in oocysts kept in old stock of Pot. dichromate solution and also in oocysts without aeration.

Earlier reports of Becker (1952) have shown the sporulation time of *E. tenella* oocysts as 48 hours. His observations were based at varying room temperature. Edgar (1955) observed complete sporulation in 18 hours.
at 29 ± 1°C. Jan-Kiewicz & Scofield (1934) and Davies & Joyner (1963) used 2% Pot. dichromate solution; Doran & Theodre (1952) used 2 - 4%, Gill et al. (1962) and Ikeda (1960) used 2.5% Pot. dichromate solution for sporulation of *E. tenella* oocysts. Currently 2% Pot. dichromate solution is used at Veterinary Laboratory, Weybridge.

The minimum time taken for sporulation in the present study is in conformity with the findings of Edgar (loc. cit.). It is further evident that 1 - 2% freshly prepared Potassium dichromate solution along with oxygen is more favourable for obtaining complete and high percentage of sporulation.

Biometrical studies of 500 oocysts of *E. tenella* at various stages of sporulation were made. The observations were based upon the diagramatic description of oocysts by Tyzzer (1929) (Plate I). Length and width of randomly selected one hundred oocysts taken at each of the unsporulated and the four sporulating stages were determined. The range of variation along with the SE for length and width are given in table IIIa. The analysis of variance was determined by 'F' test and the results are incorporated in tables IIb & IIc. The test indicates significant values at 1% level between the five groups of oocysts.
It was noticed that the cytoplasmic mass at unsporulated stage occupied whole of the oocysts and extended from the "granular layer". In stage I of sporulation there was condensation of the cytoplasmic mass in the centre. In the successive two stages granulation of the condensed cytoplasmic mass started leading to the formation of sporoblasts. In the final stage the sporoblasts developed into four sporocysts each containing two sporozoites.

Table IIa shows least difference in dimensions at unsporulated and sporulated stages. There was a significant difference between length and width of oocysts at stage I and unsporulated, stage II, stage III and stage IV oocysts. It was further observed that there existed a very slight difference in dimensions between oocysts of stage II and stage III.

The values for length and width of *E. tenella* given by Becker (*loc. cit.*), Edgar (*loc. cit.*), Tyzzer (1929) and Levine (1961) do not give the stages of sporulation and the data obtained in the present investigation do not compare well with the findings of the above workers.

Such difference in values noticed in the present study and those observed by the above workers may be due to the difference in the strain of *E. tenella*. 
involved in the present study. However, the range of variations fall within the range reported by Levine (*loc. cit.*).

Becker et al. (1955) reported that the length and width of *E. brunetti* would probably not vary during sporulation. The present study with *E. tenella* oocysts, however, indicates significant variation in their dimension at various stages of sporulation. The condensation of cytoplasm in the stage I, where the difference is more pronounced, presumably causes a shrinkage in the oocystic wall. In the next two stages, where granulation of the cytoplasm was noticed, there was an increase in size. However, this increase was not stable in the succeeding stage IV. Curiously enough, the dimensions at stage IV (fully sporulated) closely approximates with that of the unsporulated stage.

II. **Role of vitamin A in coccidiosis due to *E. tenella***.

Two groups (A and B) of laboratory-raised chicks, each consisting of ten chicks, one maintained on basal diet and the other on vitamin A deficient diet respectively, were exposed to 8,000 sporulated oocysts of *E. tenella*. Post-infection clinical symptoms were
observed daily in both the groups. A common control group, consisting of ten chicks, was maintained on basal diet without infection.

The chicks of the infected groups appeared normal until the 4th day of infection. One chick (No. 1929) of the deficient group died suddenly within 97 hours of infection without showing bloody discharge in the faeces or any other clinical symptoms. It was apparently in normal health until death. Copious bloody discharge of bright red colour appeared in the faeces of the other infected chicks on the 5th, 6th and 7th day of infection. There was no bloody discharge on the 8th day and thereafter. One more chick (No. 1974) of the deficient group died after 122 hours of the infection. This chick also was apparently healthy until death. All the infected chicks were quite active even during the acute phase of the disease. It is notable that there was little haemorrhage without death amongst the infected chicks on basal diet and control during the critical period of the disease.

Post-mortem examination of chick No. 1929 revealed initial haemorrhage on the caecal wall and that of chick No. 1974 showed profuse haemorrhage into the caecal bag (Fig. I.), from which dark red unclotted
blood oozed out when opened. It was red to mottled reddish in colour. One chick No. 1969 from group B and another (No. 1861) from group A were sacrificed on the 7th day of infection. Both were passing bloody stool, the former passing comparatively more blood. The post-mortem of caeca showed severe haemorrhage on the caecal wall of chick No. 1969 much more than that of chick No. 1861.

A natural out-break occurred amongst the original stock of chicks reared in a separate room. There were several deaths in chicks on basal and vitamin A deficient diet. One chick on deficient diet showed severe haemorrhage on the caecal wall, (Fig. II) as compared to the chicks receiving basal diet.

The surviving chicks from experimentally infected groups were passing cores, consisting of erythrocytes and tissue debris, in the faeces after 8th day of infection. The faeces were semi-solid and pale brown in colour on the 9th day of infection. Normal appearance of the faeces was observed on the 14th day of infection. Deaths after 7th day of infection could not be attributed to E. tenella infection.
These observations are in consonance with the earlier findings of Tyzzer (1929) and Mayhew (1933 & 1937) except that the infected chicks had apparently shown no loss of appetite or depression during the critical phase of the disease. A lesser degree of haemorrhage without any mortality in the chicks receiving basal diet compares favourably with earlier observations made by Allen (1932); Murphy, Hunter and Knandel, 1938; Jungherr, 1945; Taylor & Russell, 1949; Schoof, Wagner and Minier, 1954; Wachendorfer, 1957; Gylostorff, 1959; Erasmus, Scott & Levine, 1960; Gerriets, 1960 & 1961; Pellerdy, 1962; Joyner, 1963; Panda et al. 1962 & 1964.

However, the present findings are not in conformity with the findings of Jones (1934) and Waldroup et al. (1963). In the present study it was observed that the chicks on vitamin A deficient diet had severe attack of caecal coccidiosis than the chicks on basal diet when infected with same number of oocysts and reared under same managemental conditions. The increased susceptibility and severity of the disease in vitamin A deficient chicks appear to be due to the loss of natural protective function of the intestinal epithelium.
The elimination of oocysts in both the infected groups including control group are recorded in table IV. It appears from the above table that the chicks on vitamin A deficient ration passed more number of oocysts during a period of 22 days after infection, after which the faeces of both the infected group became negative for the presence of oocysts. The control group, as expected, presented a negative picture for the presence of oocysts in their faeces.

It was seen that the oocyst elimination reached the flattened peak in chicks of group A on the 9th day whereas it reacted the peak level on the 11th day of infection in chicks of group B. There occurred a decline in the oocyst elimination after these dates although daily fluctuations were noticed. Curiously enough, the chicks of group A were passing comparatively more number of oocysts on the last three days of the investigation.

Tyzzer (loc. cit.) reported that elimination of oocysts in caecal coccidiosis was somewhat irregular, probably on account of the delay in the discharge of oocysts which remained embedded in inflammatory tissues. However, by the 10th day after infection, most of the oocysts were eliminated. Allen (loc. cit.) reported
that the peak oocyst elimination was reached two days after the oocysts started appearing in the faeces. Mayhew (loc. cit.) observed peak oocyst elimination on 7th day after infection. Prasad (1963) observed that the elimination of oocysts were maximum on the 9th day after infection.

In the present study the elimination of oocysts in chicks on basal diet reacted its peak on the 9th day and in the chicks on vitamin A deficient diet on 11th day of infection. This variation in the latter group may be due to the fact that the caecal cores caused a temporary retention of the oocysts already formed and releasing the same on the 11th day. It would further appear from the table IV that there was a slight decline in the oocyst elimination on the 10th day in this group. This was also observed by Mayhew (1937).

The present findings on this aspect are in accordance with the earlier findings of Allen (loc. cit.) and Wachendorferr (loc. cit.). The fluctuations noted in the oocyst elimination after the peak elimination may be due to the cause reported by Tyzzer (loc. cit.)
Weekly weight-gain records with mean values between the three groups of chicks are given in table IIIa, IIIb, IIIc. and IIId. It indicates the pre-infection and post-infection weight-gain of chicks in all the three groups. It is evident from the data that the chicks of control group without infection weighed heavier than the other two infected groups on the date of sacrifice, though there was slight or no difference between these groups at one day old stage. The control group had maintained a progressive growth rate as measured by weight-gain during the experiment. The accompanying graph indicates that there was a depression in growth rate during the acute phase of the disease in the chicks infected with *E. tenella*. On the date of sacrifice and weeks preceding it, the infected chicks on basal diet had gained more weight as compared to the chicks on vitamin A deficient diet. The chicks on vitamin A deficient diet showed a lower weight-gain. This is in conformity with the findings of Das Gupta (1962).

Joyner and Davies (1960) observed a depression of the growth rate following infection with *E. tenella*. They reported that the birds which had received a dose of 2,000 or 5,000 sporulated oocysts showed a slight
retardation in growth rate and those with a higher
dose showed marked depression in growth rate during
the acute phase of the disease. The growth rate
was resumed after the 7th day of infection.

The present findings are in conformity with
the earlier findings of Mayhew (loc. cit.);
Bressler and Gordonk (1951); Gardnier (1954);
Joyner and Davies (loc. cit.) and Panda et al.
(1962 & 1964) and do not agree with the findings of
Allen (loc. cit.) and Waldroup et al. (1963).

It indicates that vitamin A plays an important
and beneficial role by decreasing the severity of
infection as judged by lesser mortality rate, little
haemorrhage, lower oocyst elimination and speedier
rate of growth after recovery in chicks receiving
basal diet than in chicks on vitamin A deficient diet.
It also avoids the danger of increased oocyst concen-
tration in a flock outside the host, which would
definitely play a major role in spreading of the
disease.

III. Clinical haematology:

Haematological studies were carried out in
chicks on basal diet and as well as those on vitamin A
deficient diet during the preinfection period.
41.

Post-infection haematological studies were made at weekly intervals in the chicks of group A and B infected with *E. tenella* and chicks kept as control on basal diet without infection. The data include studies on total erythrocyte count, haemoglobin percentage, erythrocyte sedimentation rate, packed cell volume, differential count of leucocytes and blood chloride expressed as sodium chloride. The results of these studies are recorded in tables V to IX. The mean values of each group are given in table X.

It appears from the tables and the accompanying graphs that during the preinfective stage the total red cell count, haemoglobin percentage, packed cell volume of chicks on vitamin A deficient diet were lower than the chicks on basal diet. There was, however, no change in the relative percentage of eosinophils, basophils and monocytes though a slight increase in heterophils and corresponding decrease in lymphocyte occurred in the chicks of group B. It is interesting to note that these chicks had a lower blood chloride value than the other group.

The chicks of group A and group B were exposed to infection on 2.5.64 i.e. when they were 21 days old. Haematological studies made on 9.5.64, as is evident from table VI revealed a decrease in red cell
count, haemoglobin percentage, packed cell volume and an increase in the erythrocyte sedimentation rate. It is interesting that during the acute phase of the disease, there occurred a marked heterophilia and lymphopaenia. In one case the relative percentage of lymphocyte was as low as 4. The haematological changes were more marked in chicks of group B than in others. There was an approximate rise of 39 mgm. and 43 mgm. in blood chloride in chicks of group A and B respectively. One chick from group A died after collection of 2 cc. blood during the acute phase. After collection of blood, the mucous membrane and face looked pale in the infected groups. Breathing difficulties with gasping was very much marked indicating anoxia in the infected groups. However, these symptoms were not seen in control group after collection of blood. This is suggestive of decrease in blood volume during the critical phase. The control group without infection on the other hand maintained a steady rise in erythrocytes, haemoglobin percentage, packed cell volume with no change in differential count and blood chloride during this period of the experiment.
The haematological studies in the next week gave an indication that the blood picture was tending towards normalcy with the exception of eosinophils. A marked eosinophilia was observed during the recovery phase. It was more pronounced in chicks of group A. There, however, persisted heterophilia although the relative lymphocyte percentage was more than in the preceding week. Heterophilia was comparatively more marked in the deficient group pointing to severity of the disease. The blood chloride value had returned to normalcy which compared favourably with the value of the control group.

By the third week after infection, the blood picture returned to normal though eosinophilia still persisted. The persistence of eosinophilia may be due to the fact that the chicks were passing oocysts at the time of examination of blood.

In the final week no marked differences were noticeable in the blood picture of group A and C. The chicks of group B were, however, showed a drop in red cell count, haemoglobin percentage and packed cell volume. The difference at this stage may be due to the effect of vitamin deficiency which hinders the absorption of nutrients and general metabolism.
43.

The haematological studies in the next week gave an indication that the blood picture was tending towards normalcy with the exception of eosinophils. A marked eosinophilia was observed during the recovery phase. It was more pronounced in chicks of group A. There, however, persisted heterophilia although the relative lymphocyte percentage was more than in the preceding week. Heterophilia was comparatively more marked in the deficient group pointing to severity of the disease. The blood chloride value had returned to normalcy which compared favourably with the value of the control group.

By the third week after infection, the blood picture returned to normal though eosinophilia still persisted. The persistance of eosinophilia may be due to the fact that the chicks were passing oocysts at the time of examination of blood.

In the final week no marked differences were noticeable in the blood picture of group A and C. The chicks of group B were, however, showed a drop in red cell count, haemoglobin percentage and packed cell volume. The difference at this stage may be due to the effect of vitamin deficiency which hinders the absorption of nutrients and general metabolism.
The above results are in consonance with the findings of Tyzzer (loc. cit.); Herrick, Ott and Holmes(1936a). The magnitude of reduction in erythrocyte count, haemoglobin percentage and packed cell volume does not agree with the findings of Herrick & Natt (1955) and Joyner and Davies (1960). They used a much higher infective dose (20,000 to 50,000 oocysts) of E. tenella in their investigation. The dose used in the present study was much less being 8,000 oocysts only. The changes in leucocytin picture favourably compares with the findings of Natt (1959) although slight eosinophilia persisted till 21st day of infection after which it rapidly decreased to normalcy.

It was also seen in the present experiment that there existed a correlation between the severity of the disease and the number of erythrocytes, packed cell volume and haemoglobin percentage as was evident in the chicks of group B. This is in conformity with the findings of Waxler (1941b) and Minchew & (1963). The increase in blood chloride during the acute phase of the disease confirms the work of Waxler (1941a), who reported that sodium chloride was released from the muscle during the critical phase and this was attributed for causing a rise in blood chloride.
However, no work was done on this aspect and hence no possible explanation could be given here.

The changes observed in leucocytic picture necessitate some discussion. Natt (loc. cit.) has not given any explanation for such changes. Code et al. (1954) reported that the increase or decrease in neutrophils and lymphocytes in circulation is governed, to some extent, by endocrine glands also though in all cases the stimulus is largely chemotactic. With the stimulation of normal adrenals with cortisone there occurs an increase in circulating neytrophils and a relative decrease in lymphocytes, eosinophils and basophils. The presence of foreign protein or the protein products set free after an inflammatory condition stimulates the bone marrow to produce more eosinophils. Biggert (1932) suggested that this increase in eosinophils was a defensive mechanism of the animal organism to protect itself against foreign protein. However, such observations were made in animals other than poultry.

It is possible that such phenomenon occurs in the chicken during the course of caecal coccidiosis in which some stimulus is released from either the adrenals or pituitary gland. E. tenella may also indirectly influence these glands during the disease.
Future probing into the subject may unravel the mystery lying behind such leucocytic changes.

IV. Histopathology:

Stained sections of the caeca of chicks (Nos. 1929 and 1974) of group B present almost similar histopathological changes after infection. These two chicks had died during acute phase of the disease after experimental infection. The degree of haemorrhage was more marked in chick No. 1974. The number and size of the schizonts were also larger in this chick. The surrounding tissues seemed to have lost their staining affinity. The cytoplasm of the second generation schizonts had taken deep stain and presented sharply contoured hyalin mass. There was practically no distinction between the outline of the parasite and the invaded cell. In some sections closely packed parasitized cells were noticed. Leucocytic infiltration into the surrounding tissue was observed. Similar changes were observed in the chicks killed on 8.5.64 from both the infected groups. But the number and size of schizonts and the resulting haemorrhage were not so pronounced in the caeca of chicks on basal diet.
In natural cases of caecal coccidiosis the size and number of the second generation schizonts were remarkably big and many. The whole gland and villi were found parasitized. There was extensive haemorrhage and destruction of tissues (Fig. 3). The epithelial cells had lost their identity and had been replaced by the parasites. In this case closely packed parasitized cells presented the appearance of a syncytium (Fig. 4). In one case extensive sloughing of parasitized cells was noticed (chick No. 1871).

The second generation of schizonts were found to develop very close to the sub-mucosa in many instances (Fig. 5). The nuclei of merozoites in the larger schizonts had taken a faint stain and found to be rather regularly distributed. The glands of Lüheber-kuhn harboured many later stages of development (chick No. 1871) (Fig. 6) and an extensive destruction and desquamation of most of the cells were seen.

In recovered chicks, however, no permanent damage was seen. The staining reaction was also normal. In some cases, from either group, one or two oocysts were seen in the tunica propria, although the chicks had apparently stopped discharging oocysts.
The study of chicks from control showed normal structures without any sign of infection.

It would appear from the above study that schizonts in the caeca of chicks on deficient diet were more in number and larger in size than those in the chicks on basal diet. Haemorrhage was also more pronounced in the sections from the former group than in the other.

The present study confirms the earlier findings of Tyzzer (loc. cit.); Mayhew (loc. cit.) and Edgar (1944). The larger and more number of schizonts in the caeca of chicks on vitamin A deficient diet might be due to the loss of protective function of the parasitized cells and possibly due to loss of certain inhibiting influence of vitamin A on the development of the parasite.
CONCLUSIONS.

I. Biology: It appears from the present investigation that the minimum time taken for complete sporulation of *E. tenella* oocyst is 18 hours at 28.5 ± 3.5 C. Freshly prepared 2% *Pot.* dichromate solution and oxygen yield a high percentage of sporulation. It seems possible that there exists a significant biometric difference in the oocysts at different stages of sporulation.

II. Role of vitamin A in caecal coccidiosis due to *E. tenella*.

The present study suggests that vitamin A plays a significant beneficial role in the course of caecal coccidiosis by minimising the severity of the disease. This was judged on the basis of lower or no mortality, little haemorrhage, lower oocyst-elimination and higher weight-gain amongst the chicks on basal diet than the chicks on vitamin A deficient diet.
III. Haematological studies revealed that there exists a correlation between the severity of the disease and changes in the blood picture.

IV. Histopathological examination of infected caeca shows that the size and number of the second generation schizonts are bigger and larger in the caeca of chicks on vitamin A deficient diet than those on normal diet. The loss of protective function of the cells and possibly absence of certain inhibiting factors on the development of the parasite in hypovitaminosis A may have attributed to such histopathological changes as mentioned above.
SUMMARY.

Infected materials were collected from natural cases of caecal coccidiosis due to *E. tenella*. The minimum time required for sporulation of the oocysts of *E. tenella* at room temperature, suitable media for their sporulation and their biometry were studied. In 18 hours complete sporulation of oocysts was observed in freshly prepared 2% Pot. dichromate solution. Oxygen and 2% Pot. dichromate were found favourable for highest percentage of sporulation. Biometrical data of *E. tenella* oocysts at different stages of sporulation revealed a statistically significant difference in their dimensions (length and width). Camera lucida drawings are furnished of these stages.

The experiment was conducted on two groups (A and B), each having 10 chicks, raised on basal diet and vitamin A deficient diet respectively. The chicks were reared on the above diets from the date of hatching. A common control group consisting of 10 chicks was maintained on basal diet without
infection during the experiment. The weight-
gain was recorded in chicks of all these groups
at weekly intervals.

The chicks of group A and B when 21 days
old were exposed to 8,000 sporulated oocysts of
E. tenella. The post-infection clinical symptoms
and post-mortem lesions were found to be in conso-
nance with the findings of earlier workers.
The severity of infection was more marked amongst
the chicks of group B with two deaths during the
acute phase than in chicks of group A. There was
no death amongst chicks on basal diet. Oocyst-
elimination reached its peak on the 9th and 11th
day of infection in group A and B respectively.
Average daily oocyst elimination was more in the
latter group save last three days. The oocysts
were eliminated by the 22nd day after infection in
both the groups. Findings of control group were
negative throughout. The chicks of control group
weighed heavier than either group A or B and
group A weighed heavier than group B at the end of
the experiment.

Haematological studies were made in the
preinfective stages on chicks of group A and B.
Post-infection haematological studies were carried
out in all the three groups at weekly intervals.
Reduction of erythrocytes, haemoglobin percentage, packed cell volume, blood volume; increased erythrocyte sedimentation rate, blood chloride, a marked heterophilia and lymphopaenia were noticed during the acute phase of the disease amongst infected groups. There was no change in blood picture in the chicks of control group during this period. Eosinophilia was observed in the second and third week after infection. It was more marked in chicks of group A than the chicks of group B. The blood picture returned to normality after 21 days of infection. Haematological changes were more pronounced in the chicks of group B than chicks of group A. A possible cause for the changes in leucocytic picture is also discussed.

Histopathological examination of infected caeca during the acute phase and after recovery were made. The number and size of the schizonts in chicks on vitamin A deficient diet were more and bigger. Changes in caeca after a natural outbreak amongst chicks have also been recorded. On many instances, the second generation schizonts were found to develop in close proximity to the submucosa.
No permanent damages were noticed in the caeca of recovered chicks though few oocysts were still found in the tunica propria.

The present study confirms the earlier opinion on beneficial role played by vitamin A in the course of caecal coccidiosis and suggests that this vital vitamin may play a significant role in prevention and control of the disease.
REFERENCES


   American J. Physiol., 96: 89-93

   J. Parasit., 41: 214-216

   Poultry Sci., 39: 565-572

18. Fisher Scientific. (1952)
   "A Manual of Colorimetric Clinical Analysis with the Fisher Clinical Electropho-

   Dtsch. tierarztl. Wschr., 67: 485-488


   Indian J. vet. Sci., 32: 240

   Arch. Geflugelk., 1: 47

   J. Parasit., 22: 264-272

24. Idem. (1936b)
   Poultry Sci., 15: 322


27. Jones, E.E. (1934)
   ibid., 85: 193-208


   exp. Parasit., 9: 243-249

   Poultry Sci., 24: 112

31. Laboratory Technique Employed at Veterinary Laboratory, Weybridge.

32. Levine, N.D. (1961)
   "Protozoan Parasites of Domestic Animals and of Man". Burgess Publishing Company, 426, South Sixth Street, Minneapolis 15, Minnesota.
34. Idem. (1932b) Ibid., 11 : 102-105
44. Idem. (1964) Ibid., 43 : 154-164


APPENDIX
OF
TABLES.
Table I

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Name of media</th>
<th>Strength of media</th>
<th>Percentage of sporulation after 48 hrs. (based on 300 observations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pot. dichromate soln.</td>
<td>0.5 %</td>
<td>79 %</td>
</tr>
<tr>
<td>2</td>
<td>Pot. dichromate soln.</td>
<td>1.0 %</td>
<td>91 %</td>
</tr>
<tr>
<td>3</td>
<td>Pot. dichromate soln.</td>
<td>2.0 %</td>
<td>92 %</td>
</tr>
<tr>
<td>4</td>
<td>Pot. dichromate soln.</td>
<td>2.5 %</td>
<td>62 %</td>
</tr>
<tr>
<td>5</td>
<td>Formaline</td>
<td>0.5 %</td>
<td>42 %</td>
</tr>
<tr>
<td>6</td>
<td>Pot. dichromate</td>
<td>2.5 %</td>
<td>7 % Without oxygenation.</td>
</tr>
<tr>
<td>Stage</td>
<td>Untransformed</td>
<td>Mean Value</td>
<td>Range of Variation</td>
</tr>
<tr>
<td>-------</td>
<td>---------------</td>
<td>------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>I</td>
<td>16.9°F</td>
<td>19.0°F</td>
<td>20.5°F</td>
</tr>
<tr>
<td>II</td>
<td>16.2°F</td>
<td>18.1°F</td>
<td>19.2°F</td>
</tr>
<tr>
<td>III</td>
<td>15.4°F</td>
<td>17.3°F</td>
<td>18.0°F</td>
</tr>
</tbody>
</table>

**Note:**
- Table II (a) shows the range of variation and average value of untransformed temperatures, along with other factors such as sample size and stages of spoilage.
### Table II (b)

**TABLE SHOWING ANALYSIS OF VARIANCE OF *E. tenella* oocysts AT VARIOUS STAGES OF SPORULATION (LENGTH).**

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Source</th>
<th>Degree of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>&quot;F&quot;</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Between groups.</td>
<td>4</td>
<td>387</td>
<td>96.75</td>
<td>12.8*</td>
<td>(*) indicates significance at 1% level.</td>
</tr>
<tr>
<td>2.</td>
<td>Within groups.</td>
<td>495</td>
<td>3718</td>
<td>5.56</td>
<td>c.f.</td>
<td>-206707</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>499</td>
<td>4105</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table II (c)

**TABLE SHOWING ANALYSIS OF VARIANCE OF *E. tenella* oocyst AT VARIOUS STAGES OF SPORULATION (Width).**

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Source</th>
<th>Degree of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>&quot;F&quot;</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Between groups.</td>
<td>4</td>
<td>164</td>
<td>41</td>
<td>-</td>
<td>(*) indicates significance at 1% level.</td>
</tr>
<tr>
<td>2.</td>
<td>Within groups.</td>
<td>495</td>
<td>2616</td>
<td>5.2</td>
<td>7.88*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>499</td>
<td>2720</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>-------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>16.5</td>
<td>8.5</td>
<td>8.5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>16.5</td>
<td>8.5</td>
<td>8.5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>16.5</td>
<td>8.5</td>
<td>8.5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>16.5</td>
<td>8.5</td>
<td>8.5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>16.5</td>
<td>8.5</td>
<td>8.5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>16.5</td>
<td>8.5</td>
<td>8.5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>16.5</td>
<td>8.5</td>
<td>8.5</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Table III

Showing mean weight gain (in grams) of chicks kept on basal diet.

Date of injection 8.9.64.

and chicks kept as control on basal diet without injection.

and subjected to different diet and injected with immunity in the.
|      | 115.33 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | Mean | 34  |
|------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 2.6.64 |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Remarks |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

**Table III(a)**

Intercept with Diazo-phenyl tetrazolium **date of injection 2.5.1664**.

Showing weight-earn in grams of chicks kept on basal diet and

<p>|       | 11.4.64 | 11.5.64 | 11.6.64 | 11.7.64 | 11.8.64 | 11.9.64 | 11.10.64 | 11.11.64 | 11.12.64 | 12.1.64 | 12.2.64 | 12.3.64 | 12.4.64 | 12.5.64 | 12.6.64 | 12.7.64 | 12.8.64 | 12.9.64 | 12.10.64 | Mean | 34  |
|-------|---------|--------|--------|--------|--------|--------|----------|----------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|-----|-----|</p>
<table>
<thead>
<tr>
<th>Date</th>
<th>18.6.54</th>
<th>19.6.54</th>
<th>21.6.54</th>
<th>26.6.54</th>
<th>29.6.54</th>
<th>30.6.54</th>
<th>31.6.54</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1</td>
<td>Initial</td>
<td>Initial</td>
<td>Initial</td>
<td>Initial</td>
<td>Initial</td>
<td>Initial</td>
<td>Initial</td>
</tr>
<tr>
<td>NOTE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE III (b)
<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Chick no.</th>
<th>Initial weight on 11.4.64</th>
<th>18.4.64</th>
<th>25.4.64</th>
<th>2.5.64</th>
<th>9.5.64</th>
<th>16.5.64</th>
<th>23.5.64</th>
<th>30.5.64</th>
<th>Final weight on 2.6.64</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1851</td>
<td>31</td>
<td>38</td>
<td>64</td>
<td>83</td>
<td>Killed on 6.5.64 for histopathological study.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1866</td>
<td>34</td>
<td>46</td>
<td>72</td>
<td>87</td>
<td>103</td>
<td>121</td>
<td>137</td>
<td>Died on 27.5.64 due to heat stroke.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1891</td>
<td>34</td>
<td>49</td>
<td>65</td>
<td>82</td>
<td>98</td>
<td>117</td>
<td>133</td>
<td>155</td>
<td>167.</td>
</tr>
<tr>
<td>4</td>
<td>1892</td>
<td>33</td>
<td>39</td>
<td>62</td>
<td>79</td>
<td>92</td>
<td>110</td>
<td>127</td>
<td>149</td>
<td>153.</td>
</tr>
<tr>
<td>5</td>
<td>1898</td>
<td>34</td>
<td>42</td>
<td>69</td>
<td>80</td>
<td>100</td>
<td>134</td>
<td>115</td>
<td>173</td>
<td>180.</td>
</tr>
<tr>
<td>6</td>
<td>1899</td>
<td>33</td>
<td>44</td>
<td>56</td>
<td>75</td>
<td>88</td>
<td>99</td>
<td>107</td>
<td>118</td>
<td>128.</td>
</tr>
<tr>
<td>7</td>
<td>1901</td>
<td>34</td>
<td>46</td>
<td>74</td>
<td>83</td>
<td>98</td>
<td>114</td>
<td>140</td>
<td>157</td>
<td>165.</td>
</tr>
<tr>
<td>8</td>
<td>1904</td>
<td>35</td>
<td>43</td>
<td>71</td>
<td>83</td>
<td>97</td>
<td>128</td>
<td>156</td>
<td>187</td>
<td>195.</td>
</tr>
<tr>
<td>9</td>
<td>1908</td>
<td>35</td>
<td>56</td>
<td>72</td>
<td>87</td>
<td>Killed on 6.5.64 for histopathological study.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1917</td>
<td>37</td>
<td>41</td>
<td>58</td>
<td>71</td>
<td>87</td>
<td>101</td>
<td>114</td>
<td>123</td>
<td>137.</td>
</tr>
</tbody>
</table>

Mean:

34 | 44.4 | 66.3 | 81 | 95.3 | 115.5 | 133.1 | 151.71 | 160.71 |
## TABLE IV

Showing average oocysts elimination per day in millions per gramme faeces in *Eimeria tenella* (date of infection infection. 2.5.1964.)

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Date of examination.</th>
<th>Days after infection.</th>
<th>Group A on basal diet</th>
<th>Group B on Vitamin A deficient diet</th>
<th>Group C on basal without infection.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.5.64</td>
<td>8th day</td>
<td>0.27330</td>
<td>0.550,250</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>11.5.64</td>
<td>9th day</td>
<td>0.31875</td>
<td>0.661,800</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>12.5.64</td>
<td>10th day</td>
<td>0.23805</td>
<td>0.387,600</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>13.5.64</td>
<td>11th day</td>
<td>0.18750</td>
<td>6.030,000</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>14.5.64</td>
<td>12th day</td>
<td>0.04215</td>
<td>1.456,050</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>15.5.64</td>
<td>13th day</td>
<td>0.17955</td>
<td>0.267,150</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>16.5.64</td>
<td>14th day</td>
<td>0.00780</td>
<td>0.791,100</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>17.5.64</td>
<td>15th day</td>
<td>0.00300</td>
<td>0.156,500</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>18.5.64</td>
<td>16th day</td>
<td>0.03195</td>
<td>0.042,150</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>19.5.64</td>
<td>17th day</td>
<td>0.01245</td>
<td>0.013,950</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>20.5.64</td>
<td>18th day</td>
<td>0.00780</td>
<td>0.109,350</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>21.5.64</td>
<td>19th day</td>
<td>0.01875</td>
<td>0.006,150</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>22.5.64</td>
<td>20th day</td>
<td>0.01395</td>
<td>0.007800</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>23.5.64</td>
<td>21st day</td>
<td>0.02025</td>
<td>0.003,000</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>24.5.64</td>
<td>22nd day</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>1.35525</td>
<td>10.38655</td>
<td>0</td>
</tr>
</tbody>
</table>

25.5.64 Faecal sample- 'A' Group negative.  
'B' Group 

26.5.64 "  
'A' " Coccidia +  
'B' " (-)  

27.5.64 "  
'A' " Sugar floatation & direct method negative.  
'B' " -do-
<table>
<thead>
<tr>
<th>Date</th>
<th>Test Type</th>
<th>Group</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.5.64</td>
<td>Faecal</td>
<td>'A'</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>sample</td>
<td>'B'</td>
<td>Negative</td>
</tr>
<tr>
<td>29.5.64</td>
<td></td>
<td>'A'</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>'B'</td>
<td>Negative</td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>69.5</td>
<td>68.5</td>
<td>67.5</td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Platelets</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Packed cell volume</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>8.3</td>
<td>8.3</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Central Phometry Form - For determination of normal picture of blood

Haematocritic data of 25 days old chickens on a standard ration of

TABLE XII.